Reversible Peptide Folding: Dependence on Molecular Force Field Used

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ABSTRACT: Temperature-dependent nuclear magnetic resonance (NMR) and CD spectra of methanol solutions of a β-heptapeptide have been interpreted in such a way that the secondary structure, a 3_{14}-helix, is assumed to be stable in a temperature range of between 298 and 393 K. This is in contrast to the results of a 50-ns molecular dynamics simulation using the GROMOS96 force field, which found a melting temperature of about 340 K. This discrepancy is addressed by further computational studies using the OPLS-AA force field. The conformational energetics of *N*-formyl-3-aminobutanamide in vacuo are obtained using *ab initio* and density functional quantum-mechanical calculations at the HF/6-31G*, B3LYP/6-31G*, and B3LYP/6-311+G* levels of theory. The results permit development of torsional parameters for the OPLS-AA force field that reproduce the conformational energetics of the monomer. By varying the development procedure, three parameter sets are obtained that focus on reproducing either low-energy or high-energy conformations. These parameter sets are tested by simulating the reversible folding of the β-heptapeptide in methanol. The melting temperature of the helix formed (>360 K) is found to be higher than the one obtained from simulations using the GROMOS96 force field (~340 K). Differences in the potential energy functions of the latter two force fields are evaluated and point to the origins of the difference in stability. J Comput Chem 21: 774–787, 2000

Keywords: peptide folding; molecular dynamics (MD) simulation; conformational analysis; *ab initio*; force field

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Introduction

In comparison to the naturally occurring α-peptides, which have been the topic of investigation in most biological contexts, unnatural peptides, such as β- and γ-peptides, have only recently received attention.[1,2] It has been recognized that β-peptides form different stable "foldamers," including helices,[1] turns,[3,4] and pleated-sheets.[4] A cyclic β-tetrapeptide has been found to mimic the biological activity of somatostatin, a cyclic α-peptide. This, coupled with the fact that β-peptides are nonproteinogetic, makes them valuable in the development of orally active drugs.[5] In this article, we report on a detailed *ab initio* conformational analysis of a β-peptide fragment, assess the development of empirical force field parameters that account for its special conformational energetics, and evaluate the performance of the force field in folding simulations.

With the availability of more powerful computers it has recently become possible to address the behavior of proteins and peptides at long time scales using computer simulation.[6] The β-heptapeptide **1** has been found to reversibly fold and unfold from a left-handed 3_{14}-helix in a molecular dynamics (MD) simulation with an explicit organic solvent (Fig. 1).[7] Based on the interpretation of temperature-dependent nuclear magnetic resonance (NMR) and CD spectra of methanol solutions of **1**, Seebach and coworkers reported that the 3_{14}-helix is stable in a temperature range of between 298 and 393 K.[8] This is in contrast to MD simulations that reported a melting temperature (T_m; the temperature at which the peptide is to 50% unfolded) of 340 K.[7b] In addition, careful examination of the reported NMR data bring into question some of the conclusions of Seebach et al. In particular, the temperature coefficients ($\Delta\delta/\Delta T$) of the NH-proton chemical shifts, which were the only data recorded up to 393 K, seem to have been overinterpreted. The values were $-2, -5, -6, -6, -4, -6$, and -5 ppb/K for residues 1 to 7, respectively.[8] The criterion used in such temperature studies is that, if the relation $\Delta\delta/\Delta T$ is linear, and has values ranging from 0 to -3 ppb/K, intramolecular hydrogen bonds are possible.[9] A value smaller than -6 ppb/K indicates absence of intramolecular hydrogen bonds, whereas values in the range -6 to -3 ppb/K do not allow for a definite conclusion in this respect. Whereas the last three residues in the 3_{14}-helix are not expected to form intramolecular hydrogen bonds (Fig. 1), residues 2 to 4 would also not be expected

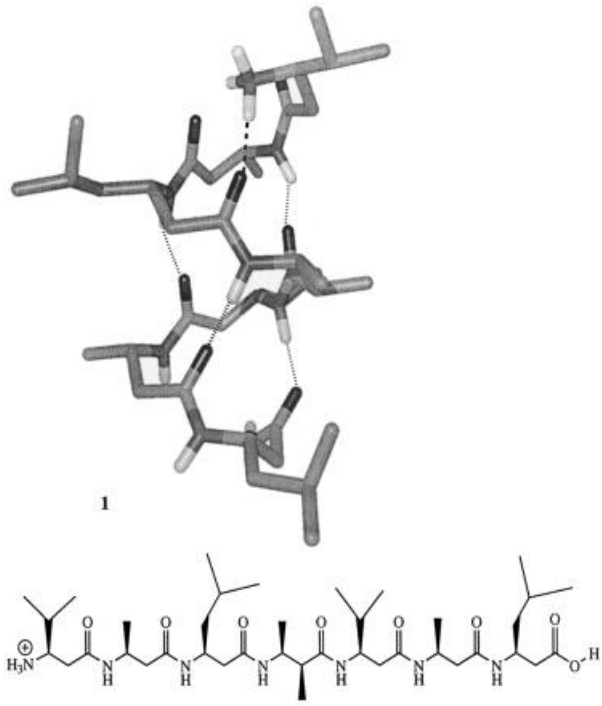


FIGURE 1. Molecular formula and three-dimensional structure of β-heptapeptide **1** derived from NMR data in methanol.

to form intramolecular hydrogen bonds according to the empirical rule. Gellman and coworkers reported X-ray and NMR data for a β-hexapeptide derived from a rigidified β-amino acid, *trans*-2-aminocyclopentanecarboxylic acid (*trans*-ACPC). The X-ray structure shows a 3_{12}-helix.[1a] The values of the temperature coefficients of the NH-proton chemical shifts reported for the hydrogen-bonded protons ranged from -4.8 to -5.4 ppb/K, whereas values for the protons not involved in a hydrogen bond (according to the X-ray structure) ranged between -8.4 and -8.6 ppb/K.[10] Even larger differences between the values of hydrogen-bonded protons (-3.5 to -5 ppb/K) and non-hydrogen-bonded ones (-15 ppb/K) were obtained for γ-peptides, which were also found to adopt a 3_{14}-helical structure.[2b] According to the empirical rules (given earlier), none of the hydrogen bonds of Gellman's β-hexapeptide and the γ-peptide would unambiguously be of an intramolecular nature. Yet, they most likely were intramolecular, because the rigidity of Gellman's β-peptide did not allow the molecule to adopt confirmations other than helical ones. It is also possible that the empirical rules quoted apply only to a limited range of chemical structures, and that the very narrow range of all the values obtained for the β-heptapeptide **1** showed

an inconclusive case. In addition, the six backbone $J_{HNC\beta}$-coupling constants of **1** measured at 353 K as ranging from 7.8 to 9.0 Hz were reproduced in an MD simulation at 360 K to within 0.5 Hz,[7d] although the MD trajectory contained only a 25% population of 3_{14}-helical conformations. Therefore, we are not convinced that there are five intramolecular hydrogen bonds forming a stable helix over the entire temperature range quoted by Seebach et al. To address this uncertainty, we undertook the calculations reported in this article.

The reliability of an MD simulation is to a larger extent dependent on the accuracy of the interatomic interaction function or force field used. There are a number of force fields specially designed for and known to perform well in simulations of biomolecular systems. Among these is the GROMOS96 force field[11] with which the reversible folding of **1** was initially discovered. In this report the OPLS all-atom (OPLS-AA, optimized potentials for liquid simulations) force field[12] is extended with the necessary torsional parameters optimized for fragments of β-peptides and tested in similar simulations of the reversible folding of β-heptapeptide **1**. Furthermore, the energetics of interactions involved in the folding process are compared for both force fields and compared with the results of *ab initio* calculations, giving insight into the possible origins of the differences between the simulation results.

The OPLS-AA (all-atom) force field is the successor to the OPLS united-atom force field. Simulations of biomolecular systems using the new all-atom representation have not yet been reported. However, the conformational analysis of succinic acid and its monoanion have been investigated successfully in the gas phase and in aqueous solution.[13] The attempt to parameterize carbohydrates has revealed technical difficulties.[14] Similar difficulties were also encountered in the current work. Nevertheless, the OPLS-AA parameters used in static (0 K) calculations have been reported to perform well in reproducing carbohydrate crystal structures measured at room temperature, in comparison to other force fields.[15] Furthermore, the OPLS-AA force field was the first to reproduce the free energy of solvation of amines and amides with different methyl substitutions.[16] Other force fields, even ones including polarization terms, failed to reproduce the trends in free energy.[17]

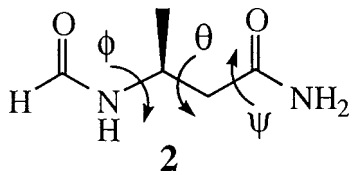
The force field parameters of the OPLS-AA force field have been optimized to reproduce physical properties, including the heat of vaporization and the density of pure liquids, and also to reproduce the energetics of key conformations in the gas phase. This has been done for a series of monofunctional compounds including alkanes, alcohols, amines, and amides, and for many more functional groups with which most biomolecular compounds can be described. The systematic and uniform development of the OPLS-AA parameter set and its success in studies of small polyfunctional molecules make it attractive for use in testing larger biomolecular systems.

In comparison, the GROMOS96 force field consists of a parameter set that has grown and improved continuously over the years. Initially, the parameters were developed by adjusting energies and distances, calculated in the gas phase at 0 K, of water–protein and protein–protein interaction complexes to reproduce condensed-phase data.[18] As in the OPLS approach, the parameters describing the solvent models of the GROMOS96 force field, such as the SPC water model,[19a] CCl_4,[19b] DMSO,[19c] and $CHCl_3$,[19d] have been developed to reproduce the physical properties of pure liquids. Further improvements were done by using the same procedure to develop parameters for alkanes, which were subsequently tested in calculating the free energy of hydration.[20] These parameters were also found to improve the simulation of hen egg-white lysozyme. This protein was simulated with GROMOS96 and the results were compared with the data obtained from a simulation conducted with an older version of the GROMOS force field.[21]

Computational Details

AB INITIO CALCULATIONS

The conformational energetics of a typical fragment of the β-heptapeptide **1** consisting of *N*-formyl-3-aminobutanamide **2** were studied (Fig. 2). Based on the conformational energetics provided by



2

FIGURE 2. Molecular structure of β-amino-amide derivative **2** studied with force fields and *ab initio* calculations. ϕ is defined by the C—N—CH—CH dihedral angle, θ is defined by the N—CH—CH—C dihedral angle, and ψ is defined by the CH—CH—C—O dihedral angle.

this study the force field parameters were developed. At the beginning, the complex conformational space spanned by the three dihedral angles, ϕ, θ, and ψ (Fig. 2), was searched to a limited extent. A rotational profile for the rotation of the central N—CT—CT—C torsional angle ϕ was calculated in 30° increments at the B3LYP/6-311+G* level of theory.[22] Starting from the minima obtained, further possible orientations of the planes represented by the two amide groups were investigated using the HF/6-31G*, the B3LYP/6-31G*, and the B3LYP/6-311+G* levels of theory. Using the conformational search utility of the BOSS program,[23] with the optimized dihedral angle potential parameters for β-peptide fragments, no further conformations with an energy of <8 kcal/mol were found. The two sp_2-centers of the amide group were kept rigid in all Z-matrices, which is the default for the corresponding optimizations using the OPLS-AA force field. Using this restriction, the structures and energies obtained with the *ab initio* methods are not affected. However, the strong out-of-plane bending of the NH bond observed in some force field-optimized structures is prevented with this restriction, which facilitated the fitting of the force field torsional parameters.

Structures were optimized in internal coordinates using Schlegel's optimizer,[24] as available in the GAUSSIAN-94 program package,[25] which was used for all electronic structure calculations. The 6-311G basis set is the smallest basis set optimized using the simplest electron correlation method (MP2), and was therefore chosen in combination with the B3LYP density functional method.[26] In what follows, all electronic structure calculations are referred to as *ab initio* methods, although density functional methods are not exactly *ab initio* in character.

The structures of interaction complexes were obtained by full optimization at the HF/6-31G* level of theory. Subsequent single-point calculations were done at B3LYP/6-311+G** and used to determine the interaction energy.[27] The interaction energy was calculated as the difference between the energy of the complex and its monomers without applying the counterpoise correction for the effects of basis set superposition error (BSSE), which has been reported to be appropriate at the B3LYP level of theory.[27d]

FORCE FIELD PARAMETER DEVELOPMENT

The conformational energetics from the *ab initio* calculations enabled optimization of a limited set of torsional parameters for the OPLS-AA force field. All force field parameters describing monofunctional amides and ammonium anions were initially assigned to the β-peptide fragment. As found in the parameterization of carbohydrates[14] and diacids[13] it may be necessary to use different torsional parameters for quartets of the same AMBER atom types,[28] W—X—Y—Z, in different molecules. When optimizing torsional parameters for polyfunctional molecules, it is not clear, a priori, which torsional parameters have to be optimized and how much structural information (i.e., number of *ab initio*-calculated structures and energies) must be provided for the fitting. Because *ab initio* calculations for a polyfunctional molecule such as **2** are rather time-consuming, the parameter development is carried out in stages. At the beginning, only a limited set of *ab initio*-calculated structures is taken into account. Thus, for torsional parameter set A, 12 rotamers obtained from a rotational profile around the N—CT—CT—C torsion were considered. With this limited set of conformations it was possible to optimize the parameters of the N—CT—CT—C and the N—C—CT—CT torsional types, whereas all other torsional types were assigned to the standard OPLS-AA torsional parameters. With the set of 22 *ab initio*-calculated structures and energies, it was also possible to optimize the parameters for the O—C—CT—CT torsional type. This led to parameter sets B and C, where for set B the conformations within a range of 6 kcal/mol (17 conformations) were included, and for set C all 22 conformations were included. Excluding the higher energy conformations gives a higher weight to the low-energy conformations during the parameter fitting, so that low-energy conformations can be expected to be reproduced better.

The torsional parameter fitting was accomplished using the FITPAR program.[29] Geometry optimizations were started from the *ab initio*-calculated structures and were performed with the BFGS algorithm, as available in the BOSS program.[23] In cases where a conformation did not represent a local minimum of the force field the optimization was repeated with a dihedral angle held fixed at the torsional angle value of the corresponding *ab initio*-calculated structure.

MD SIMULATIONS

Three 50-ns MD simulations were performed using the GROMOS96 package of programs and the OPLS-AA parameter set. The dynamics of the β-heptapeptide in methanol were studied at three

temperatures, 360 K, 380 K, and 400 K, at 1-atm pressure, and with periodic boundary conditions. Three 10-ns MD simulations were performed at lower temperatures, 300 K, 320 K, and 340 K. The temperature and pressure were maintained by weak coupling to an external bath.[30] The initial structure of the peptide for all simulations was the 3_{14}-helical fold, as determined experimentally. The system consisted of the β-heptapeptide and 1055 methanol molecules in a rectangular box. A twin-range cutoff of 0.8 nm per 1.4 nm was used for all nonbonded interactions, whereas the long-range interactions were updated every five steps. The shortest distance between the peptide and the wall of the box was initially 1.4 nm. Configurations of the system were stored every 0.5 ps. The simulation conditions and the analysis of the trajectories were identical to the simulations conducted earlier using the GROMOS96 force field.[7a]

For methanol, the OPLS united-atom parameter set was used in combination with a rigid geometry.[31] The simulation conditions were found to reproduce accurately the experimental density and heat of vaporization of methanol. The subroutines of the GROMOS96 simulation package that calculate the potential energy terms and the corresponding forces were adapted to treat the OPLS-AA parameters correctly. The scaling factor of two for the 1,4-electrostatic interactions and the harmonic potential energy terms for bond stretching and angle bending were implemented in the GROMOS96 code.[11] To verify the correct implementation of the OPLS-AA parameters in the GROMOS96 package, parallel single-point calculations were conducted on the initial peptide structure with the BOSS simulation package, used for the development of OPLS-AA, and with the modified GROMOS96. The individual contributions to the total potential energy were verified to be identical in both programs.

Results and Discussion

AB INITIO CALCULATIONS

The relative energies and the dipole moments as a function of the three dihedral angles are presented in Table I. The conformations were ordered according to an increasing N—C—C—C(O) dihedral angle θ. Variations in the relative energies between the HF and B3LYP results ranged up to 2 kcal/mol. Nevertheless, orderings of the relative energies were quite similar. The global minimum was the sc$^-$ conformation **2t**, which was stabilized by a hydrogen bond (Fig. 3). This conformation was also

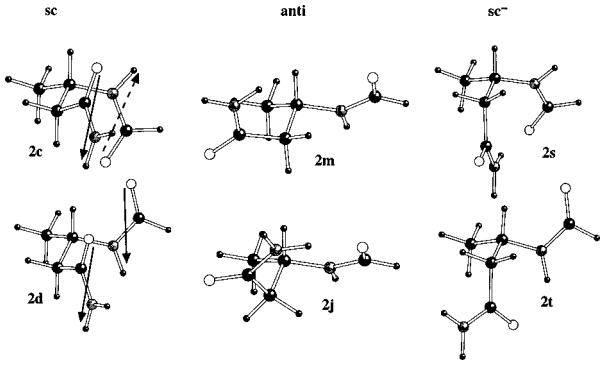


FIGURE 3. Selected conformations of *N*-formyl-3-aminobutanamide **2** ordered in columns with a synclinal, anti, and synclinal$^-$ arrangement of the N—CT—CT—C(O) dihedral angle θ.

detected in experiments conducted in chloroform solution of a very similar derivative of **2**, *N*-acetyl-3-aminobutanoic acid-*N*′-methylamide,[32] as well as in two other *ab initio* conformational analysis done on similar β-peptide fragments.[33] All other sc$^-$ conformations showed energies of at least 6 kcal/mol higher energy than **2t**. Of the five sc conformations, **2c**–**g**, only **2d** had a relative energy of >2 kcal/mol. The anti conformations, **2l**–**o**, were all >2 kcal/mol in energy relative to **2t**. The anti conformation lowest in energy, **2m**, was not a minimum. It was necessary to hold the angle θ fixed during optimization (Table I). The minimum of the potential energy well was shifted to an anticlinal arrangement of dihedral angle θ in which a hydrogen bond was formed (**2j**, Fig. 3). Only with this hydrogen bond is an anti conformation lower in energy than an sc conformation. Excluding all the hydrogen bonded conformations that can be expected not to be populated in a protic solvent, as found in the studies of succinic acid in aqueous solution, one ends up with a simple characterization of conformational energetics. The sc conformation (**2c**) was lower in energy than the anti (**2m**), which was itself lower than the sc$^-$ conformations (**2s**, **u**, **v**).

This observation is consistent with Seebach's prediction that the conformation adapted in the helix of β-heptapeptide **1** must be a preferred one.[1c] However, the sc conformation, **2d**, in which the two amide groups are arranged as in the helix, is not a minimum of **2**. It was necessary to keep the angles θ and ψ fixed during the optimization. Due to the parallel arrangement of the two amide dipoles, **2d** had the largest dipole moment of 7.2 Debye. The energy of conformation **2d** was 4.3 kcal/mol, about 2 kcal/mol higher in energy than a non-hydrogen-bonded conformation, **2m**, and about 2.6 kcal/mol

TABLE I.

Relative Energies, Dipole Moments, and Selected Dihedral Angles of β-*N*-Formyl β-Alaninamide Conformations at Various Levels of Theory.

	HF[a]	B3LYP[a]	B3LYP/6-311+G*					OPLS-AA Set A[b]					GROMOS96	Set B[b]	Set C[b]
	energy[c]	energy[c]	Energy[c]	Dipole[d]	θ[e]	ϕ[e]	ψ[e]	Energy[c]	Dipole[d]	θ[e]	ϕ[e]	ψ[e]	energy[c]	energy[c]	energy[c]
2a[f]	9.09	8.36	8.03	2.66	0.0[g]	84.0	107.6	7.81	2.42	0.0[g]	78.5	107.6[g]	7.06	7.04	7.26
2b[f]	3.88	4.29	3.66	1.84	30.0[g]	66.1	80.1	3.37	1.63	30.0[g]	62.4	80.1[g]	3.62	3.07	3.19
2c[f]	1.59	2.37	1.69	0.68	60.0[g]	64.1	40.1	0.99	0.58	60.0[g]	59.2	41.9	1.76	0.88	1.06
2d	4.77	5.13	4.34	7.23	60.0[g]	−153.8	48.4	4.83	7.92	60.0[g]	−154.7	48.4[g]	3.71	4.78	4.85
2e	2.15	2.01	1.88	4.84	47.9	−170.7	−78.1	4.00	4.13	50.4	−155.1	−86.7	−0.05	2.78	2.99
2f	2.04	1.22	1.13	4.21	66.6	−108.0	−172.8	2.40	4.73	87.4	−100.0	161.5	2.00	1.82	2.53
2g	1.33	1.75	1.58	2.32	52.9	49.8	66.9	0.95	0.56	58.5	58.5	42.0	1.56	0.83	0.97
2h[f]	3.72	3.95	3.29	1.37	90.0[g]	61.9	18.9	2.79	1.91	90.0[g]	61.7	31.3	4.31	3.00	3.12
2i[f]	5.41	5.47	4.63	1.32	120.0[g]	59.8	−11.6	4.15	1.86	120.0[g]	62.4	−31.1	5.60	4.59	4.86
2j	1.93	1.43	0.95	5.05	131.8	−70.2	112.3	2.22	4.92	118.0	−70.9	114.6	4.70	2.03	2.39
2k[f]	3.41	3.87	3.15	0.92	150.0[g]	57.8	−29.0	2.49	0.96	150.0[g]	58.4	−30.6	4.47	1.91	2.36
2l[f]	3.71	4.11	3.46	1.23	180.0[g]	61.8	−50.9	3.03	1.65	180.0[g]	61.5	−41.7	1.87	1.72	2.35
2m	2.84	3.25	2.19	1.74	180.0[g]	−79.4	92.4	3.66	1.67	180.0[g]	−81.0	63.3	2.88	0.24	0.92
2n	3.08	3.55	2.96	5.58	180.0[g]	−73.2	−22.7	3.72	6.37	180.0[g]	−79.8	−22.7[g]	4.04	2.53	2.93
2o	7.32	6.74	6.65	6.01	180.0[g]	60.6	50.0[g]	6.87	6.76	180.0[g]	60.9	50.0[g]	6.26	5.65	6.13
2p[f]	6.53	6.39	5.71	3.31	−150.0[g]	62.1	−82.8	5.78	2.79	−150.0[g]	62.8	−64.6	6.48	4.53	5.13
2q[f]	5.72	4.44	4.29	5.21	−120.0[g]	55.8	−103.9	4.12	4.76	−120.0[g]	58.1	−101.0	7.28	2.39	3.02
2r[f]	7.10	5.11	5.35	5.52	−90.0[g]	84.5	−148.6	4.45	5.26	−90.0[g]	82.5	−147.7	9.00	2.72	3.42
2s[f]	7.70	6.06	6.28	4.78	−60.0[g]	97.0	176.0	5.92	4.54	−60.0[g]	94.8	176.6	7.87	4.82	5.51
2t	0.00[h]	0.00[i]	0.00[j]	6.02	−60.0[g]	−132.1	34.5	0.00	6.02	−60.0[g]	−132.4	31.7	0.00	0.00	0.00
2u	9.24	8.73	8.65	6.24	−77.3	83.0	−78.0	7.81	6.77	−77.3	75.4	−78.0[g]	5.73	6.93	7.20
2v[f]	9.75	8.38	8.41	3.79	−30.0[g]	95.6	142.4	8.52	3.51	−30.0[g]	91.5	141.6	7.30	7.09	7.49
								RMSD[k]					RMSD[k]	RMSD[k]	RMSD[k]
							All 22 conformations	0.8					1.5	1.1	0.9
							Conformations <4 kcal mol^{-1}	1.0					1.1	0.8	0.7

[a] Using 6-31G* basis set.
[b] OPLS-AA force field with a torsional parameter set optimized for β-peptide fragments.
[c] Energies in kcal mol^{-1}.
[d] Dipole moments in Debye.
[e] Angles in degrees.
[f] Conformation shown in rotational profile of Figures 4 and 7.
[g] Angle held fixed at the given value during the geometry optimization.
[h] Absolute energy of **2t** is −453.822344 au.
[i] Absolute energy of **2t** is −456.53483 au.
[j] Absolute energy of **2t** is −456.6676768 au.
[k] RMSD for the energy difference between the B3LYP/6-311+G* and the force field calculated relative energies of the corresponding column.

higher than the corresponding sc (**2c**) conformation. This shows that, although an sc conformation is preferred for **2**, the arrangement of the two amides as in the helix is high in energy. The intramolecular hydrogen bonding of the helix will, however, compensate for this high energy.

TORSIONAL PARAMETER DEVELOPMENT

The parameters of the initial optimized torsion set A are given in Table II. At the time this fitting was done not all 22 conformations were calculated at the highest level of theory. Thus, the global minimum, **2t**, was not included in the initial fitting (Fig. 3). The relative energies obtained with the fitted parameters, however, reproduced the finding that **2t** was the global minimum and that, in most sc conformations, **2c**–**g**, the anti conformations **2l**–**o**, and the sc^- conformation were in the range of the *ab initio*-calculated energies (Table I). Only one conformation (**2e**) showed a discrepancy of >2 kcal/mol and only three conformations (**2f**, **j**, **m**) deviated by >1 kcal/mol. The root-mean-square deviation (RMSD) in energy for all 22 conformations was 0.8 kcal/mol (Table I) and the average discrepancy was 0.6 kcal/mol, which is close to the value of 0.5 kcal/mol reported for diacids. This shows that, even for a limited set of conformations used for torsional parameter fitting, good agreement between force field and *ab initio*-calculated energies can be obtained. This indicates further that the electrostatic interactions in the molecular mechanics model do not differ very much from the ones reproducing the *ab initio* data exactly.

The RMSD between the *ab initio* and the force field-calculated relative energies over the 22 conformations of **2** was 1.1 kcal/mol for parameter set B, and 0.9 kcal/mol for parameter set C (Table I). These values are both higher than the RMSD obtained for parameter set A, which is 0.8 kcal/mol. All relative energies obtained with parameter set B are smaller than those obtained with set C. For anti and sc^- conformations **2l**–**v**, the *ab initio*-calculated relative energies were larger than those obtained with parameter set C, which demonstrates that set C reproduced the high-energy conformations better than set B. Parameter set A performed even better for these high-energy conformations. This explains its low RMSD. However, when only energies of conformations with an *ab initio*-calculated relative energy of <4 kcal/mol are compared, the RMSDs of parameter sets B (0.8 kcal/mol) and C (0.7 kcal/mol) are both smaller than that obtained for parameter set A (1.0 kcal/mol). This shows that the optimization of an additional torsional type for parameter set C improves the fitting for low-energy conformations. It can be concluded that parameter set C should be used in future investigations of β-peptides. The influence of the different parameter sets on the stability of the β-heptapeptide **1** was studied further with MD simulations at different temperatures (*vide infra*).

The GROMOS96 force field parameter set was also tested for reproducing the conformational energetics of the β-peptide fragment **2**. The parameters for β-peptides were assigned in analogy to the ones from α-peptides. These have not been optimized to reproduce any conformational energetics in the gas phase. The RMSD obtained for the 22 conformations of **2** was 1.5 kcal/mol, not much higher than that obtained for the optimized OPLS-AA torsional parameter set A (0.8 kcal/mol, Table I). Larger deviations were mainly found for sc^- conformations.

TABLE II.
Sets of Torsional Angle Parameters for OPLS-AA Force Field Corresponding to the Potential Energy Function[a]:

$$E_{\text{torsion}} = \sum_i \frac{V_1^i}{2}[1 + \cos(\phi + f_1)] + \frac{V_2^i}{2}[1 + \cos(2\phi + 2f_2)] + \frac{V_3^i}{3}[1 + \cos(3\phi + 3f_3)]$$

	N—CT—CT—C			N—C—CT—CT			O—C—CT—CT		
	V_1[b]	V_2[b]	V_3[b]	V_1[b]	V_2[b]	V_3[b]	V_1[b]	V_2[b]	V_3[b]
Set A	−5.450	−0.101	−1.161	4.878	0.0	−0.988	0.0[c]	1.166[c]	0.0[c]
Set B	−4.019	0.0	0.0	3.403	0.442	0.18	0.626	−0.307	0.0
Set C	−4.555	0.0	0.0	4.345	0.388	0.203	0.881	−0.318	0.0

[a] ϕ is the dihedral angle, and f_1, f_2, and f_3 are all zero.
[b] Given in kcal mol^{-1}.
[c] Optimized for propanamide.[12a]

Looking into the details of the conformational energetics obtained with the newly fitted parameter sets reveals some general problems. The sc conformation, **2d**, represents an arrangement found in the helix of **1**. Because the stability of this helix depends on it, the energy of this conformation relative to others is of special interest. The energy difference between conformation **2d**, where the two amide dipoles are aligned parallel, and sc conformation **2c** (Fig. 3), with the dipoles aligned in the opposite direction, was at *ab initio* level 2.6 kcal/mol, whereas the torsional parameter sets of the OPLS-AA force field give 3.8 (set A and C) and 3.9 (set B) kcal/mol. The GROMOS 96 force field obtained a smaller energy difference of 0.9 kcal/mol, which stabilized the helix of **1**.

There are two possible sources for such discrepancies. First, in the OPLS-AA force field, the 1,4-electrostatic interactions were scaled by a factor of two. This was recognized to lead to an imbalance, which cannot be corrected for with the torsional parameters.[14] Second, the non-bonded parameters were optimized to reproduce the physical properties of pure liquids of monofunctional compounds. This optimization led to partial charges with which the molecular dipoles were overestimated by about 10% to 15%. Consequently, the interaction energies of the gas phase were also exaggerated in comparison to *ab initio*-calculated ones (*vide infra*). The inclusion of explicit polarization in a molecular force field would lead to smaller partial charges, reducing the problems of excessive electrostatic interactions and eliminating the need to scale 1,4-electrostatic interactions.[34]

MD SIMULATIONS

In Figure 4, the RMSD of the backbone atom positions of residues 2–6 of the simulated structures (parameter set A) from the NMR-model structure is shown as a function of time. An RMSD value of <0.1 nm indicates a conformation very close to the NMR-derived one. Significant deviations from the NMR model were found only at temperatures of >360 K. Therefore, simulations at <340 K were stopped after 10 ns. Analysis of the structures col-

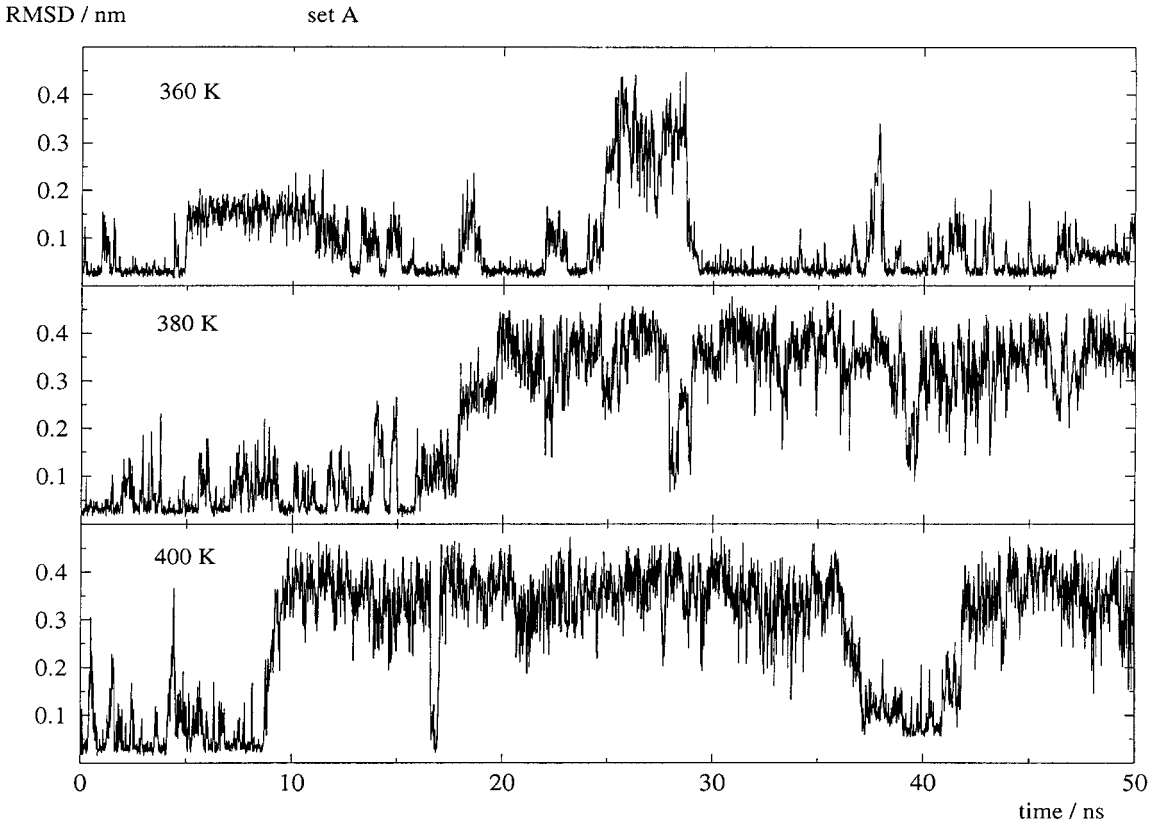


FIGURE 4. Backbone atom positional RMSD from the (helical) reference structure as a function of time in simulations at different temperatures for torsional parameter set A. Structures with an RMSD of <0.1 nm have unequivocally the same fold as the reference structure to which they are compared.

lected in 10-ps intervals of the trajectories obtained during 50 ns reveals that the helix was present up to about 80% at 360 K, to about 33% at 380 K, and to about 22% at 400 K. The trajectory obtained at 380 K shows that the β-peptide unfolded after 15 ns and refolded only once thereafter, whereas, at 400 K, only two incidences of refolding occurred during 50 ns. Because the peptide is mostly stable at 360 K and does not refold to a helix with a lifetime longer than a few picoseconds at 380 K, one can estimate that the melting temperature is between 360 K and 380 K. This is significantly higher than the melting temperature of 340 K reported for the GROMOS96 force field.

The RMSD values obtained for parameter sets B and C are shown in Figure 5. The simulation at 360 K using parameter set C showed a mostly stable helix. The highest values for the RMSD were only 0.1–0.15 nm, indicating that the helix was only partially unfolded. The simulation run at 370 K shows an unfolding event after 4 ns and a refolding after 12 ns. The fold obtained after 12 ns was for only a short time a complete helix and the next unfolding occurred after about 13 ns. At 380 K one unfolding event occurred and, at up to 20 ns, no refolding was observed. This indicates that the melting temperature for parameter set C was also in the range of about 360 to 380 K.

The torsional parameter set B, which yielded a too-small relative energy for most conformations of **2**, shows that the helix unfolded after 300 ps at a temperature of 360 K and after 6 ns at 340 K. For the remainder of both simulations the peptide stayed unfolded. The simulation run at 350 K showed an RMSD value that was always <0.2 nm, indicating that only partial unfolding occurred during the total simulation time of 20 ns. This example shows that analysis of a single simulation of insufficient length can lead to the wrong conclusions. The simulation at 350 K would indicate that the helix is mostly stable at this temperature. However, the simulations at the three different temperatures clearly indicate that, in using parameter set B, the peptide unfolded at a temperature lower than 360 K, whereas the simulations using parameter sets A and C indicate a melting temperature at >360 K. Parameter set B underestimated the relative energies of high-energy gas-phase conformations, as shown earlier. This indicates that the energy of these high-energy

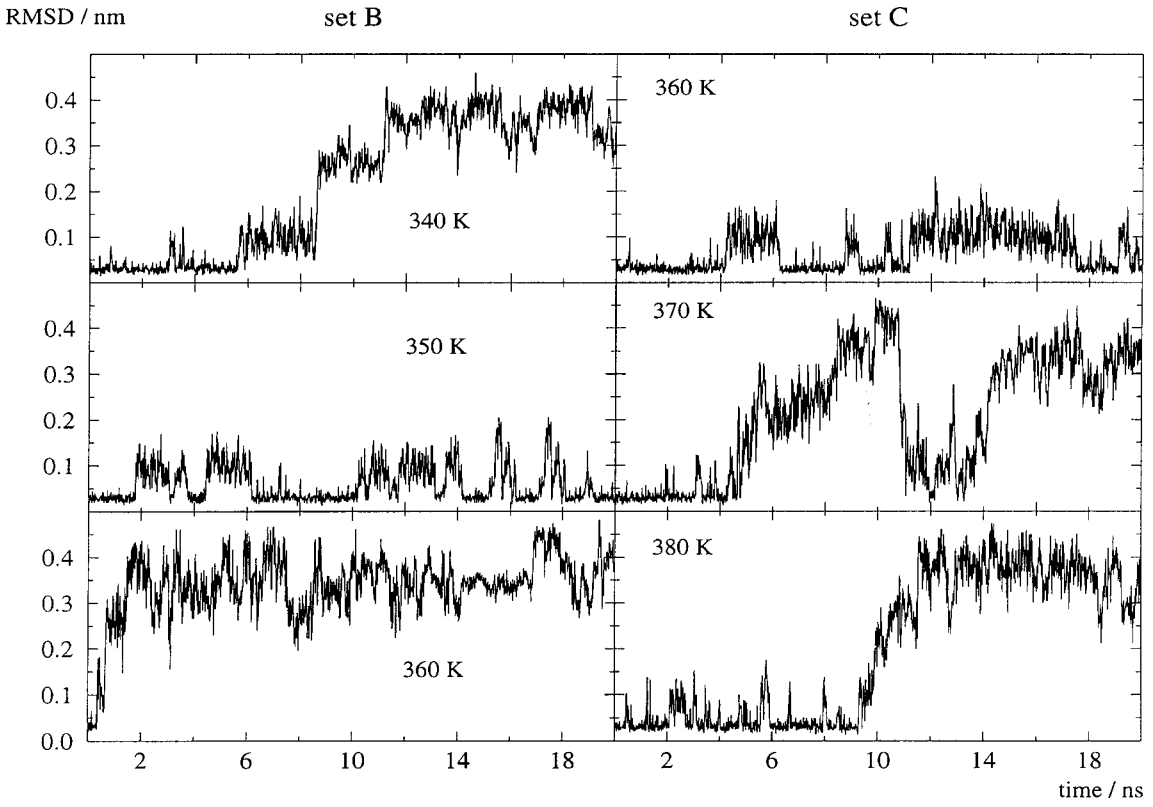


FIGURE 5. Backbone atom positional RMSD from the reference structure as a function of time in simulations at different temperatures for torsional parameter sets B and C.

conformations influenced the melting temperature of the peptide.

Parameter set C performed better in reproducing the *ab initio*-calculated relative energies of low-energy conformations than parameter sets A and B. The estimated melting temperature obtained for parameter set C was similar to that estimated for parameter set A, which best reproduced the *ab initio*-calculated high-energy conformations of **2**. This indicates that parameter set C would be preferred over sets A and B.

When visually comparing the RMSD plots obtained at high temperatures (380 and 400 K) for torsional parameter sets A and C to the ones obtained with the GROMOS96 force field the following becomes apparent. The time intervals in which the peptide was unfolded (RMSD >0.1 nm) were, on average, shorter, and refolding occurred more frequently in the simulations conducted with the GROMOS96 force field. This shows that the dynamics with the OPLS-AA force field were slower and that sampling of the conformational space was therefore less complete. The RMSD plots given in Figures 4 and 5 show that, during unfolding events, plateaus of constant RMSD values can be found, which lasted from several hundreds of picoseconds to nanaoseconds. Inspection of the structures of the different plateaus indicates that, during these time periods, structures with a different number of hydrogen bonds were found (Fig. 6, top), compatible with the stepwise mechanism of unfolding proposed by Seebach et al.[8] However, cooperative folding events also occurred in the simulation from structures with very high RMSDs within a very few picoseconds (Fig. 6, bottom). A detailed cluster analysis comparing the trajectories obtained with the two force fields, GROMOS96 and OPLS-AA, will be reported separately.[35]

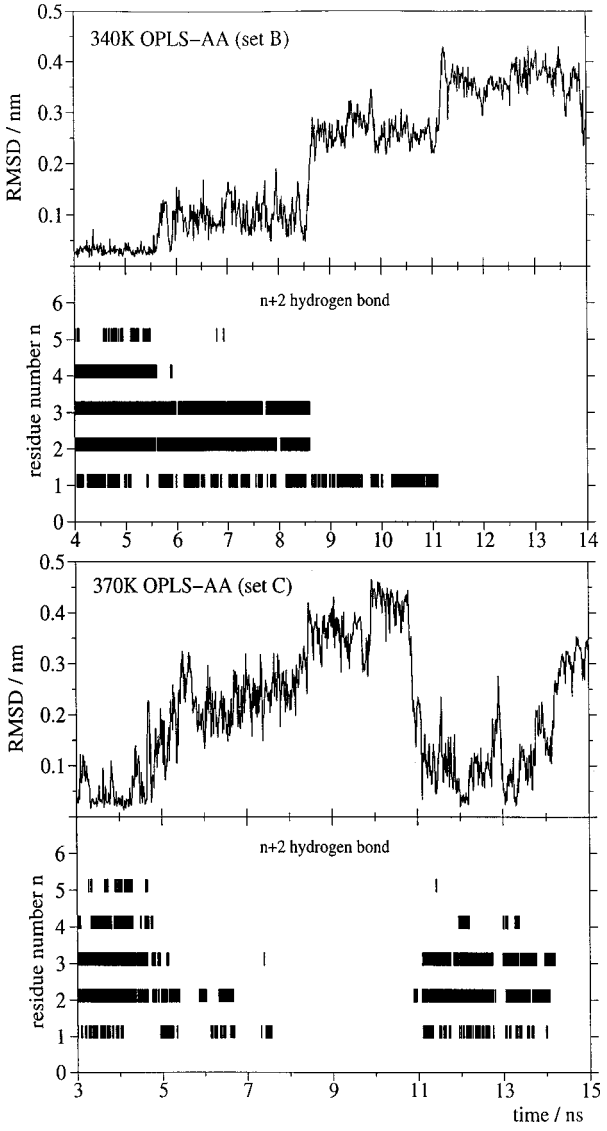


FIGURE 6. Backbone atom positional RMSD from the reference structure as a function of time compared with the hydrogen bonds occurring between residues *n* and $n + 2$. A hydrogen bond is indicated when the distance between a nitrogen atom and a carbonyl oxygen atom is <0.3 nm.

FORCE FIELD PARAMETERS AND INTERACTION ENERGIES

To find the origin of the different melting temperatures obtained for the GROMOS96 and the OPLS-AA force fields, key parameters and interaction energies were compared. In Figure 7, the peptide partial charges used in the GROMOS96 force field are compared with the ones used in the OPLS-AA force field. The GROMOS96 force field generally used smaller charges than the OPLS-AA force field. As a consequence, the 1,4-electrostatic interactions did not need to be scaled down. Both force fields used charges on atoms of the carbonyl group, which made this group electrically neutral. Such small,

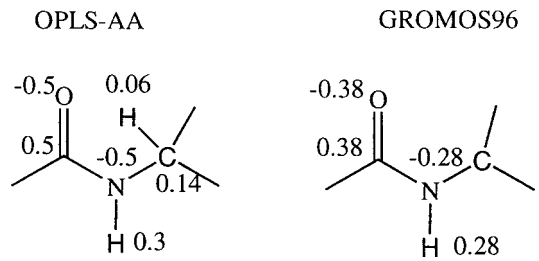


FIGURE 7. Comparison of partial charges (units e) used in the force field calculations.

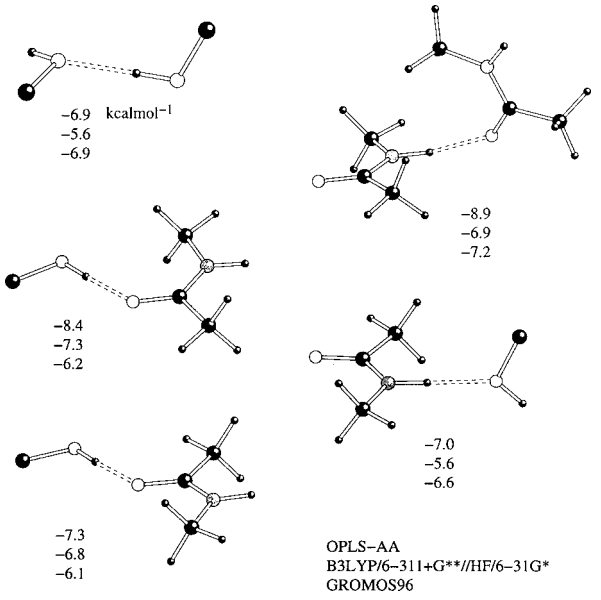


FIGURE 8. Structures and energies (kcal/mol) of key interactions in the folding process compared at different levels of theory.

neutral groups of atoms are called charge groups in the GROMOS96 nomenclature. The charges assigned to atoms of the N—H bond also form a charge group in GROMOS96, whereas the OPLS-AA force field spreads its charges over the adjacent carbon and hydrogen atom. The charge on this hydrogen atom (0.06e) is the one assigned to all aliphatic hydrogen atoms. The charge on the carbon atom is then chosen to obtain a neutral charge group.

The effect of the differences in charges together with the different Lennard–Jones parameters is demonstrated in the interaction energies for the *N*-methylacetamide dimer, the methanol dimer, and the complexes of methanol and *N*-methylacetamide (NMA) in Figure 8. Both force fields obtained the same gas-phase interaction energy (−6.9 kcal/mol) for the methanol dimer. This is not surprising, because both force fields were adjusted to reproduce the experimental heat of vaporization and the density of methanol. This interaction energy was about 20% larger than the one obtained at the *ab initio* level. This is typical for force field parameters of a condensed-phase force field, which have to account implicitly for many-body effects.[36]

The OPLS-AA-calculated NMA–NMA interaction energy (−8.9 kcal/mol) was also about 20% higher than that calculated *ab initio* (−6.9 kcal/mol). In contrast, the GROMOS96 force field obtained an interaction energy for the NMA dimer of −7.2 kcal/mol, close to the *ab initio* value, and 1.7 kcal/mol smaller than the one obtained using the OPLS-AA force field. With all calculation methods the NMA–NMA interaction was stronger than the methanol–methanol interaction. This is consistent with the stronger polarization in amides, which is reflected in the much larger dipole moment for NMA (3.9 D) than for methanol (1.8 D).

For the methanol–NMA complex, two structures were found where methanol acted as a donor and NMA as an acceptor. OPLS-AA gave an interaction energy of −8.4 kcal/mol, which is 1.1 kcal/mol larger when the OH bond is close to the nitrogen atom. Almost no energy difference between these two complexes was obtained using the GROMOS96 force field. The interaction energies calculated using GROMOS96 were 2.2 and 1.2 kcal/mol smaller than the ones obtained with the OPLS-AA force field. The *ab initio*-calculated interaction energies were between the ones of the two force fields, which shows that the GROMOS96 force field underestimated this interaction energy in comparison to the OPLS-AA force field, by 1.1 and 0.7 kcal/mol, respectively.

Only one interaction complex was obtained in which methanol acted as an acceptor and NMA as a donor. At the *ab initio* level, and also for the OPLS-AA force field, NMA is a better acceptor than a hydrogen bond donor, as is commonly found for amides. The GROMOS96 force field detects the opposite. Both force fields yielded a larger NMA donor interaction energy in comparison to the *ab initio* method.

In contrast to the torsional parameter development, a force field describing the liquid phase is expected not to reproduce the interaction energies obtained from gas-phase *ab initio* calculations, but rather to overestimate them by about 10% to 20%. With such increased interaction energies, the OPLS-AA force field has been shown to reproduce the heat of vaporization and densities of liquids for a series of amides and alcohols, and many other compounds.[12] In comparison to the interactions calculated with the OPLS-AA force field, the *ab initio*-calculated interaction energies do not explicitly consider many-body effects observed in the liquid phase. The *ab initio*-calculated energies therefore give the lower limit of what the dimer interaction energy of a force field representing the liquid phase should be. Accordingly, the interaction energies obtained with the OPLS-AA force field are all larger in magnitude than those obtained with the *ab initio* method. Thus, not only are implicit polarization effects accounted for in the parameters adjusted to reproduce the physical properties of pure liquids

(NMA, methanol), but also in the nonbonded parameters arising from the combinations of these parameters for separate species (NMA–methanol complex).

Unlike the OPLS-AA force field, the interaction energies obtained with the GROMOS96 force field were not consistently larger than the interaction energies obtained at the *ab initio* level. The NMA–methanol interactions were underestimated in comparison to the *ab initio*-calculated ones by 1.1 and 0.7 kcal/mol. The NMA–NMA interaction, in comparison to the OPLS-AA force field (2.0 kcal/mol), was only slightly larger (0.3 kcal/mol). Consequently, the experimental pure liquid properties for NMA were not reproduced accurately. At a temperature of 373 K the calculated heat of vaporization of neat NMA was 22% too low in comparison to the experimental value, and the density is too low by 5%.[37]

With interaction energies of the various complexes all overestimated compared with the gas phase, the OPLS-AA force field (parameter sets A and C) obtained a melting temperature of >360 K. It can therefore be speculated that a change of the GROMOS96 parameters, such that the interaction energies of all complexes are overestimated with respect to the gas phase, could also lead to a higher melting temperature. Such a change could involve an increase of the charges of the carbonyl group. It has been shown that an increase in all the amide charges in GROMOS96 by a factor 1.125 would lead to the correct sign, in comparison to the experiment, for the change in free energy of transfer of tryptophan analogs from chloroform to water.[38] This increase in charges also improves the pure liquid properties calculated for NMA, yielding a heat of vaporization and density 6.9% and 2.7% lower than the experimentally determined values, respectively.[37]

The GROMOS96 force field was able to detect the correct fold for a β-heptapeptide in a simulation started from a completely extended conformation.[7a] This is not the only example in which the GROMOS96 force field is able to predict the correct fold for a β-peptide.[39] The simulations conducted with the OPLS-AA force field show relatively rare incidences of refolding, and the folds obtained have very short lifetimes. It is therefore unlikely that such folds can be predicted within a reasonable computational time using the latter force field in a simulation started from an arbitrary conformation. This, however, does not consider that the exact time scale for the folding is not known for the β-heptapeptide. It seems like the reversible folding in simulations of β-peptides obtained by the GROMOS96 force field would be favorable, due to the faster dynamics relative that obtained with the OPLS-AA force field. The difference in rate with which the peptide conformations are interconverted in solution is based on the special balance of the interaction energies between intrasolute, solute–solvent, and solvent–solvent interactions.

Conclusions

In this study the development of three torsional parameter sets (A–C) for the OPLS-AA force field was described. These were tested in simulating a β-heptapeptide in methanol at different temperatures. For parameter set C, the largest number of conformations was used for the fitting, and more (three vs. two for set A) torsional types were adjusted for a β-peptide fragment. Parameter set C is advisable for future investigations because it obtained the smallest RMSD for reproducing the *ab initio*-calculated energies in a range up to 4 kcal/mol, and obtained MD simulation results similar to those of set A, which best reproduced the high-energy conformations of the β-peptide fragment.

The stability of the β-peptide was found to depend on the conformational energetics of the β-peptide fragment used to develop the torsional parameters. Similar to α-peptide, this indicates that the stability of a peptide depends on the conformational energetics spanned by the ϕ and ψ angles of a dipeptide. The interaction energies involved in folding or unfolding processes were calculated for the OPLS-AA and GROMOS96 force fields as well as for the B3LYP/6-311+G**//HF/6-31G* method. Comparison of the force fields showed that stronger interactions were obtained for the OPLS-AA force field. These stronger interactions originated from a parameterization aiming to reproduce the pure liquid properties of the compounds under consideration, leading to greater peptide stability. Because the GROMOS96 force field does not follow this parameterization procedure completely, one can conclude that this force field is somewhat loosely based on theory. The OPLS-AA force field strictly follows this parameterization procedure and the results obtained from the MD simulations displayed a higher melting temperature (360 to 380 K) than the GROMOS96 force field (~340 K). In agreement with Seebach's conclusion, it is therefore possible that the helical structure is largely intact at temperatures of about 353 K.[8]

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